Holographically corrected microscope with a large working distance

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Abstract

We present a design for a microscope with a simple, high numerical aperture objective lens. The large amounts of aberrations present in the system are removed by recording a point source image hologram. The resultant instrument has a large working distance (> 0.17m) and a moderate field of view over a limited bandwidth. We demonstrate the application of this device to imaging sub-micron details inside a vacuum.

Keywords: Holography, microscope, high resolution, micromachining, microlithography, aberration correction.

1. Introduction

In order to resolve small details, high numerical aperture imaging optics are required. Fast objectives will have a large amount of spherical aberration, so the aperture is usually minimized, which means that the working distance must also be made very small. If a large working distance is required, expensive multi-element combinations are necessary. An alternate solution is to use a single-element system and remove the aberrations holographically. We use holography to correct a simple, large numerical aperture lens and achieve diffraction limited imaging. The benefits of this design are the ability to observe objects in real-time, at high magnification, in situations where close proximity to the sample is undesirable or otherwise impossible, such as samples under vacuum, in toxic/radioactive environments or in microbiological applications.

Holography was originally developed as a means for correcting the spherical aberration present in electron microscopy and has since been applied to many optical systems including microscopes[(1-,12)]. Two distinct types of holographic microscopy have been investigated previously. The first[5,6] involves recording holograms of objects through good quality microscopes and observing the images in a separate step, with the hologram doing little to correct for aberrations present in the system. In the second design type[7,10], a hologram of the object is recorded through a microscope of any optical quality, with the aberrations removed by using a phase conjugate reconstruction. However, this method still requires a good quality microscope to observe the reconstructed images, and as with the other designs, a new hologram must be recorded for each object. In the design presented here, the aberrations of the microscope, in imaging a pinpoint of light, are recorded holographically. This hologram can then be used to remove the same aberrations present with any object imaged by the optical system.

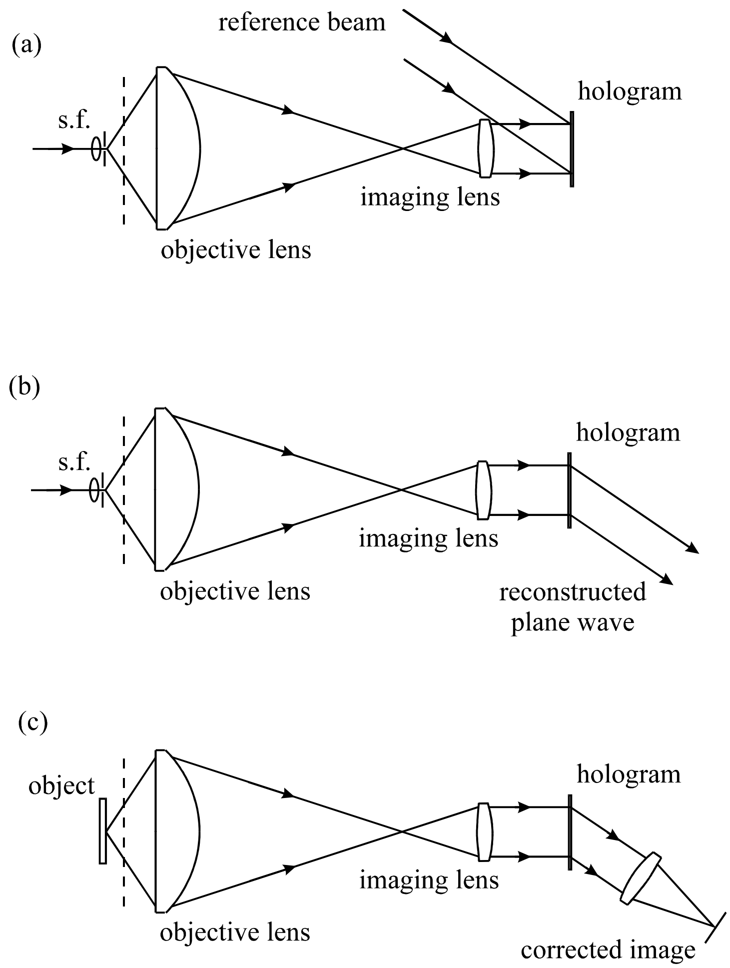

Figure 1: (a) Recording. Laser light is spatially filtered to illuminate the objective lens which is then imaged onto the film. A hologram is recorded with a plane wave reference beam. The dashed line indicates the possible inclusion of a vacuum window. (b) Reconstruction. With the set-up unchanged, the object beam will reconstruct the original reference beam. (c) Imaging. Light from an object placed at the pinhole position will reconstruct the reference beam which can be focused to form an unaberrated image.

The basic operation of a holographically corrected refracting microscope is shown in Figure 1. The recording procedure begins with a spatial filter which illuminates a simple objective lens [Fig 1a]. While the size of the pinhole in the spatial filter does not effect the final resolution of the microscope, it must be sufficiently small that the objective is evenly illuminated. The light is focused by the objective and gathered by an imaging lens which collimates the light and images the objective lens onto the plane of the hologram. This imaging condition increases the field of view, when using an aberrated objective, by defining a particular phase shift associated with each point on the lens for any field point[13]. The recording media (in our case plate film) is aligned perpendicular to the object beam, and the hologram is recorded with a diffraction limited plane wave.

The processed hologram is returned to the original recording position for reconstruction [Fig. 1b]. With the spatial filter illuminating the objective once more, the light will pass through the optical system, reconstructing the plane wave reference beam. If the spatial filter is replaced by an object illuminated with light of the recording wavelength, light from a point corresponding to the original position of the pinhole will reconstruct the reference beam *with object information retained* [Fig 1c]. If this beam is focused, an unaberrated image of the source point is obtained. To an extent, the aberrations of nearby points will be similar to that of the source point, so the hologram is able to correct for a finite field of view.

2. Experimental Evaluation

An experiment was conducted using an inexpensive Pyrex plano-convex objective lens (f = 165 mm, D = 108 mm) illuminated by a spatial filter, consisting of a 60X microscope objective and a 0.5 μm pinhole, at a working distance of 175 mm. For a more dramatic demonstration, the spatial filter was placed inside a vacuum chamber, illuminating the objective through a thick glass window (indicated by the dashed line in Figure 1). In this configuration, the focused beam has a paraxial spot size 240 mm in diameter and over 1900 waves of spherical aberration, along with several waves due to the poor surface quality of the lens and window. A diffraction limited imaging lens (f =100 mm, D = 36 mm) was placed at the circle of least confusion to form a 25 mm diameter image at the plane of the film, 110 mm away. The intensity distribution across the image beam changes dramatically either side of the image plane, so to ensure an even intensity (for the most efficient hologram), the plate is placed perpendicular to the object beam. A hologram was recorded with a diffraction limited reference beam incident on the plate from an angle of about 30^o. Having the reference beam come in from an angle results in a diffracted beam, on reconstruction, with an elliptical profile. This does not result in any aberration being present in the final beam, just a change in aspect ratio which can be corrected digitally. The plate film was held in kinematic holder which made it possible to replace the hologram to its original position after processing. For this experiment we used a CW, frequency doubled Nd:YAG (λ = 532 nm) laser and bleached Agfa 8E56 plate film to record phase transmission holograms with typical diffraction efficiencies (ratio of 1^{st} diffracted order power to input power) of 70-90%.

After exposure and processing, the hologram was returned to the holder, and the chamber containing the spatial filter was evacuated to 10^{-4} Torr. With the set-up essentially unchanged, the spatial filter illuminated the objective lens forming the original object beam which reconstructed the reference beam at the hologram. The fidelity of the reconstructed beam was tested by examining the focal spot [Fig. 2a], and by forming an interference pattern against the original reference beam [Fig. 2b]. From these results we can conclude that the on-axis correction was better than $\lambda/10$.

a. b.

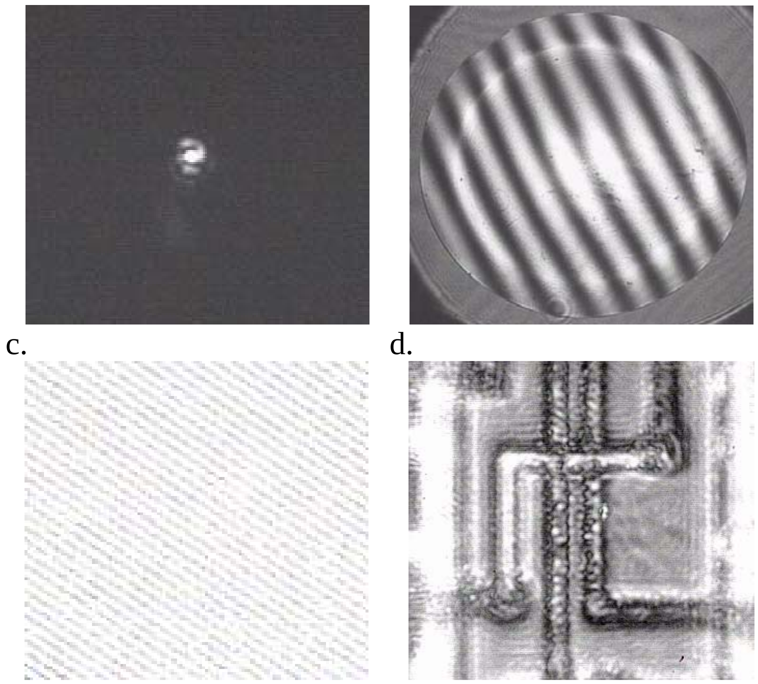

Figure 2: (a) The focal spot of the reconstructed beam. (b) The interference pattern of the reconstructed beam against a plane wave showing $<\lambda/10$ wavefront error. (c) The image of a sinusoidal grating (1130 lines/ mm) in transmission. (d) The image of a microcircuit in reflection. The tracks are about 3 μm wide.

The spatial filter was replaced by a sinusoidal amplitude grating (with a grating spacing of 0.885 μm) illuminated from the rear by laser light passing through a rotating diffuser to remove speckle. The chamber was then evacuated once more, with the light from the object reconstructing a diffracted beam which was focused to form an image of the grating [Fig. 2c]. The low visibility of the fringes is a result of the operation near the Rayleigh limit of the microscope.

For a reflecting object, a slightly different set-up was required in order to illuminate the object. We recorded a new hologram with a half-silvered plane mirror placed just before the imaging lens, at a 45^{o} angle to the incoming beam. This made it possible, on reconstruction, to introduce a beam which travels backwards through the system to provide on-axis illumination to a reflecting sample. Figure 2d shows an image of an integrated circuit (under vacuum) with details of <3 μm clearly visible. The resolution of such fine details under vacuum and at a working distance of >170 mm should have many benefits. In particular, the microchip industry where this microscope makes it possible to view such processes as plasma deposition/etching of microscopic features *in situ*.

These measurements show that even with such large initial errors, diffraction limited, on-axis correction and imaging is possible. However, since no off-axis aberrations are recorded, there will be a reduction in correction with field angle. The field of view and depth of focus were determined by translating the position of the spatial filter (no longer under vacuum) and re-examining the diffracted beam interferometrically. We found the diffraction limited field of view to be ±10 μm with a ±2 μm the depth of focus. The effect of these uncorrected off-axis aberrations is apparent in the large-field images shown in Figures 3a&b. Although the field of view is small, by scanning a sample under the on-axis recording point a large object can be viewed at the highest possible resolution. This technique is commonly used in confocal and electron microscopy in order to increase the effective field of view.

a. b.

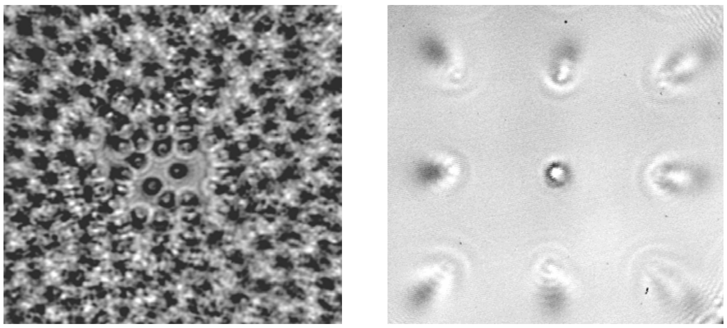

Figure 3: Images showing the effect of uncorrected off-axis aberrations over a large field of view. (a) Blood cells. (b) An array of 5 μm holes.

As well as a narrow field of view, this microscope also has a small operating bandwidth. The hologram records the phase error of the system at a particular wavelength, and as such, there will be a wavelength dependent error. The final, corrected phase error ϕ_2, is related to the initial phase error, ϕ_1 and the read/write wavelengths (λ_2 and λ_1 respectively) by:

$$\phi_2 = \frac{|\lambda_2 - \lambda_1|}{\lambda_2}\phi_1 \tag{1}$$

A reconstruction of the point-source was performed using Argon-ion (λ = 514 nm) and green HeNe (λ = 543.5 nm) lasers. The residual aberration at these wavelengths was found to be 68 and 41 waves respectively, which agrees well with this simple theory. Given the large initial wavefront error in this system, perfect imaging can only occur at the recording wavelength. However, the bandwidth could be increased, quite simply, if the objective lens characteristics were optimized to reduce the amount of spherical aberration initially present in the system

3. Alternative Designs/Modes of Operation

Although we have demonstrated the correction of a microscope with a simple refracting objective, several other designs are possible. First, a Fresnel lens or Fresnel/Gabor Zone Plate could serve as the objective element. The microscope set-up is the same as used for a conventional lens, but with this design much larger numerical apertures are possible while the overall weight of the instrument is decreased.

A second possible design could incorporate a reflecting, conic objective, used in an off-axis arrangement as shown in Figure 4. In this arrangement there will be large amounts of off-axis aberrations present in the system. However, these wavefront errors will be recorded and corrected holographically, just as with the on-axis aberrations in the refracting microscope. The advantage of using a reflecting objective is that the microscope operation can be extended into UV wavelengths for greater resolution.

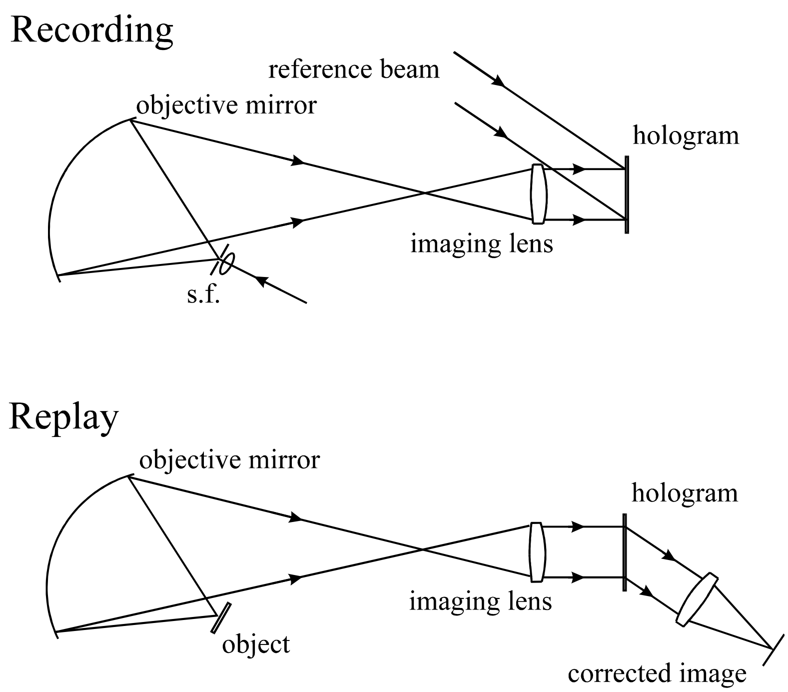

Figure 4: Reflecting microscope. The recording and replay set-ups are shown for a holographically corrected microscope with an off-axis objective element.

We have demonstrated how this microscope can be used for producing magnified images of objects, though it is possible to use the same instrument for photo-reduction and micromachining. If a phase-conjugate of the original reference beam is incident on the hologram, a phase-conjugate of the object beam will be reconstructed making it possible to create high numerical aperture, diffraction limited focal spots. We have used this technique to machine pinholes less than 1μm in size in metal foils under vacuum using low power pulsed lasers. Furthermore, images can be projected through the system for high resolution photo-lithography or photo-reduction.

Conclusion

We have investigated the design of a holographically corrected refracting microscope with a simple, high numerical aperture objective operating at a large working distance. A point source of light is used to record an image hologram of the objective which makes it possible to correct for the aberrations present when viewing any object. The simple, inexpensive device provides a useful field of view over a limited bandwidth while making it possible to view objects in situations where traditional microscopes fail. The microscope can be used as an inexpensive method of high resolution image reduction and micro-lithography and can be adapted to incorporate conic reflectors, zone plates, or Fresnel lenses as the objective elements.

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Acknowledgments

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